

The University of Chicago

**THE DIFFERENTIATION OF
HEPATIC AND PANCREATIC TISSUES
OF THE CHICK EMBRYO
IN CHORIO-ALLANTOIC GRAFTS**

A DISSERTATION SUBMITTED TO THE
FACULTY OF THE DIVISION OF THE
BIOLOGICAL SCIENCES IN CANDIDACY FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF ZOOLOGY

1932

By

RUTH HOLTON SANDSTROM

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THE DIFFERENTIATION OF HEPATIC AND PAN-
CREATIC TISSUES OF THE CHICK EMBRYO
IN CHORIO-ALLANTOIC GRAFTS¹

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THE differentiation of the early hepatic rudiment into apparently normal liver tissue when grafted to the chorio-allantoic membrane has been reported by several workers. Hoadley (1925, p. 150) makes note of it in his grafts of heart level of 37-hour chick embryos, Hunt (1929) found it in grafts of node level taken in the advanced head-process stage, and a similar finding was reported by Willier and Rawles (1931a) in their study of grafts of whole blastoderms in early stages of development. In general, these observations show that the hepatic tissue which differentiates from grafts of parts of the early blastoderm is histologically normal.

On the other hand, Murphy (1916) and Willier (1924) found that adult liver tissue when grafted to the chorio-allantoic membrane ceased to grow and underwent extensive degeneration. The former found in his grafts of adult chicken liver that there was a lack of growth and an intense infiltration of the tissue by leucocytes. Willier alluded to this same phenomenon in his paper on the effects of thyroid grafts on the chick embryo, pointing out that grafts of liver tissue from newly hatched and adult chickens will not grow when grafted to the chorio-allantoic membrane. Instead, apparently the only parts that persist are the bile ducts.

With these results in mind, experiments on hepatic tissue were made to determine the time at which the liver, having ceased to grow and differentiate, becomes degenerated, and if possible the underlying causes for this change.

The work on the liver came naturally to include the pancreatic tissue, and a comparable study of that gland was undertaken also, to ascertain whether at a definite period it too would show a similar

¹ I wish to express my appreciation to Dr. B. H. Willier for the many valuable suggestions and criticisms which he has so generously given throughout the work.

cessation of growth and a subsequent degeneration, in chorio-allantoic grafts. In 1924 Danchakoff reported normal differentiation of pancreatic tissue transplanted from donors of 7 days' incubation, and other workers (Willier and Rawles, 1931a; Hunt, 1929; Hoadley, 1925), using pre-rudimentary stages, also found pancreatic tissue which was apparently normal in its differentiation. Reports other than these on the behavior of early embryonic material have not been found in the literature; consequently, the experiments on pancreatic tissue recorded here form a more extended group than do those performed in connection with the work on the liver.

MATERIAL AND METHODS

Embryos of Single-Comb White Leghorn chickens were used for both the hosts and the donors. The type of isolation used in the experiments varied in accordance with the age of the donor. In the very early stages of development (i.e., up to 3 days) complete isolation of the rudiments appeared impossible, so that three different regions of approximately the same level were grafted independently: (a) a piece of the floor of the gut just large enough to include the rudiment of the liver, (b) a piece of the roof of the gut large enough to include the diverticulum of the dorsal pancreas, and (c) a ring of gut tissue of about the same size including both of these rudiments. The isolated pieces were not measured, but were for the most part less than 1.0 mm. square. In the case of older donors, the pieces either consisted of the primordium alone or included also a small part of the adjoining region of the gut. The purpose of this last isolation was to test the influence of the gut tissue on the development of the gland rudiment. In the series of pancreatic isolations, donors of 10 or more days' incubation were handled in two ways. In most cases the gland was implanted in fragments because of its size, but in a few instances the entire gland was grafted for purposes of comparison. The instruments used to make these isolations were fine ear knives, one with a single cutting edge and the other with a double spearlike edge.

In the experiments on the liver, some of the donors were incubated for 32-35 hours (18-20 somites) and others for 3, 4, 5, and 6 days. The implants for the pancreatic series were taken from donors in-

cubated from 3 to 19 days. The original method of grafting, described by Willier (1924, p. 68), was used, with the modifications already noted. The implants were placed on hosts of 8 or 9 days' incubation and were allowed to remain until the host was 18 days old, when they were removed and prepared for histological examination.

Both Bouin's solution and Zenker-formal were used as fixatives, and the sections were cut at 6 or 8 micra from paraffin blocks. The hepatic grafts were stained in Heidenhain's iron hematoxylin and counterstained with Orange G. This combination was also used as the routine stain for the pancreas sections. From several of the grafts, however, test sections were set aside and stained with special dyes to determine whether an apparently normal pancreas was physiologically active. For these tests a few sections from each slide of the serial were mounted separately; the object was to obtain as representative an area as possible for the reaction of the dye. Stains specific for zymogen granules, which occur in the acinar tissue, and for the alpha and beta cells of the island tissue were employed. Neutral gentian demonstrates the zymogen granules well but the stain fades very quickly, consequently Mallory's azan stain and Bensley's combination of aniline acid fuchsin and methyl green were the stains mainly used since they are specific for both types of tissues. By the former method the alpha and beta cells are differentiated with red and yellow granules, respectively, and the zymogen granules are red; whereas with the latter, the zymogen granules and the granules of the alpha cells are red and the beta granules are blue. The azan stain is a particularly good one, for it has little tendency to fade.

For purposes of comparison, the normal development of these glands was followed; through the fifteenth day of development for the liver, and in the case of the pancreas up to the time of hatching. The staining methods used in the experimental work were also employed in these series of normal development.

STAGES IN THE DEVELOPMENT OF THE HEPATIC AND PANCREATIC RUDIMENTS AT THE TIME OF IMPLANTING

The liver.—The hepatic rudiments used for transplantation were taken from donor embryos incubated 3, 4, 5, and 6 days, thus covering the early developmental changes in the gland.

The liver first appears as a ventral outpushing of the gut at the 22-somite stage, just as the right and left wings of the splanchnopleure come together in the median line. In the 35-somite chick, as the gut continues to close a second ventral outpushing appears, a little posterior to the first. At 68 hours the posterior diverticulum develops a bud which represents the primordium of the gall bladder. At first the two anlagen are distinct, and each gives off branches which unite into a close network of hepatic cords.

At the end of the third day of development, the hepatic cords of the two rudiments have anastomosed with each other to form a single organ, which is subsequently bilobed. As the two liver buds come together, they surround the vitelline veins, and by the fourth day, incipient blood capillaries can be seen in the mesenchyme between the hepatic cords. At this stage the gall bladder is a small but definite pouch, although it does not begin to store bile until the sixth day of incubation. During the fifth and sixth days, the solid columns of hepatic cells continue to grow and anastomose until the organ consists of a compact mass of characteristic polyhedral cells with large nuclei and occasional vacuoles in the cytoplasm. The solid cords acquire narrow lumina which are the bile capillaries. The blood capillaries become sinusoidal and connect with the vitelline veins.

The pancreas.—The pancreatic tissues used in the transplantations were taken from embryos varying in age from 3 to 19 days of incubation. This was found necessary since in this gland the changes occur more gradually than they do in the liver. In the very earliest operations only the dorsal diverticulum was grafted, whereas after the ventral diverticula appear all three were transplanted.

According to Lillie (1919), the pancreas develops from one dorsal and two ventral diverticula. The dorsal one arises first from the roof of the gut at the level of the anterior intestinal portal in the 35-somite chick. Following this, on the fourth day (96 hours) the right and left ventral outpushings arise from the common hepatic diverticulum near its union with the duodenum. Clara (1924), in a report on the pancreas of the bird, gives a summary of the constitution of the gland and his statements agree with those of Lillie. Among the birds that Clara included were the chick, the sparrow, the pigeon, and the duck; but since his interest was largely histological, he did

not investigate the embryological foundations of the gland. He refers to three lobes, two principal ones and one very small "spleen segment," which he thinks correspond to the three original anlagen.

During the fourth and fifth days of development, the primary out-pushings grow, become branched, and extend into the surrounding mesenchyme, where they form distinct glandular masses. At this time a complete isolation of the ventral rudiments is impossible because of their close association to the common bile duct. By the close of the sixth and on the seventh day of incubation the dorsal diverticulum has extended into the mesenchyme surrounding the ventral rudiments, forming the foundations of the adult organ. At this stage, with care it can be isolated from the liver. In an embryo of 7 days' incubation the gland consists of a few tubules imbedded in mesenchyme; the tubules are connected to the gut by one or more ducts.

By the tenth day the pancreas has become a compound tubular gland with many branched tubules terminating in small acini. Activity of these acini is demonstrated by zymogen granules which, with Mallory's azan stain, stand out as sizable red particles. At 12 days the tubules are more branched and the stroma is so condensed that the gland has a more compact appearance. The Islands of Langerhans make their appearance at this time, first as isolated alpha and beta cells scattered between the tubules and acini. The island tissue is easily recognizable in sections treated with azan stain. The many beta cells contain small yellow granules whereas the comparatively few alpha cells exhibit large red granules. By the fifteenth day the islands have increased in number and size so that the whole gland assumes the appearance of the adult organ.

THE GRAFTS

A total of 198 grafts has been examined histologically: these include both liver and pancreatic tissues in seven types of isolations.

In general, the blood supply of the grafts is somewhat variable. The theory was that this condition might bring about histological changes in the tissues of the grafts, but actually it was found that such changes depended on the age of the tissue rather than upon the richness of the blood supply from the host.

I. GRAFTS FROM A PIECE OF THE FLOOR OF THE GUT LARGE
ENOUGH TO INCLUDE THE LIVER RUDIMENT

Donors with from 15 to 28 somites furnished the isolations for this series of experiments. The part to be grafted, approximately 1.0 mm. square, was taken from the floor of the gut at the level of the anterior intestinal portal, and an effort was made to avoid including the side walls of the gut. Nineteen grafts were sectioned and in 9 of them small areas of liver, gut, and pancreas were observed. In addition, 2 of the 9 cases contained bits of heart tissue. Of these 9 grafts 2 were from donors of 15 somites and the remaining 7 were from donors of 16, 18, 21, 23, 25, 26, and 27 somites. In an abstract reporting the early part of this work (Holton, 1929), it was stated that neither gall-bladder nor pancreatic-tissue occurred in the grafts. Although the sections were not stained selectively for pancreas in the early work, further study has shown that the tissue was present and an epithelial vesicle identified as gall bladder occurred in numerous cases.

Hepatic tissue is identified as a compact mass of tubules and sinusoids, connected either with the gut tissue of the implant or with the gall bladder when that structure is present. The sinusoids which are united with the host blood vessels contain blood corpuscles. The tubules consist of cells with large, rounded, darkly staining nuclei and vacuolated cytoplasm, thus conforming cytologically to normal liver tissue of a corresponding age. The small lumina surrounded by these cells correspond to the bile capillaries of the normal tissue. In the resting cells the cytoplasm and nuclei are normal, but many of the cells appear to be in stages of mitosis, indicating growth in the graft.

The pancreas appears in only 2 of the 9 grafts (23 and 28 somites), but in these instances it is identified as an irregular mass of tubules connected by ducts to the gut tube. The tubules may be acinous, but with all the stains tried they appear to be simply lumina with cuboidal cells surrounding them, for the characteristic zymogen granules, which normally appear on the tenth day, are entirely lacking. The absence of the zymogen indicates that the tissue is not functionally normal 9 days after its transplantation to the host. Between the tubules a great deal of island tissue is present. This tissue

is identified at first by the peculiar bluish granules of the beta cells following the iron-hematoxylin stain. In the azan stain the beta cells are recognized by their yellow granules and the alpha cells by their red granules. These observations seem to place the development of the islands in the graft slightly in advance of those in a normal pancreas, for definite islands are usually not found before the fifteenth day.

2. GRAFTS FROM A PIECE OF THE ROOF OF THE GUT LARGE ENOUGH
TO INCLUDE THE DORSAL PANCREATIC RUDIMENT

The 19 grafts of this series were obtained from donors of 64-90 hours (35-44 somites) and consisted of small pieces of the roof of the gut at the level of the anterior intestinal portal, and large enough to include the dorsal pancreatic diverticulum. Some were taken just before, and others just after, the appearance of the primordium. All showed development of pancreatic tissue except one (68-hour donor with 35 somites). Of these transplants, 6 were grafts from donors of 89-90 hours, after the appearance of the primordium, 6 were from donors of about 75 hours, and 7 came from donors of 64-68 hours, before the actual appearance of the outpushing. In 4 of the 6 grafts in which the pancreas was isolated shortly after its appearance, small amounts of hepatic tissue were identified. As in the foregoing series, the pancreatic tissue consisted of normal islands and tubules connected with the gut by ducts.

3. GRAFTS OF A RING OF THE GUT TUBE INCLUDING ALL OF THE
LIVER AND PANCREATIC RUDIMENTS

These grafts were taken from donor embryos of 15-38 somites and consisted of cross-sections of the gut at the level of the existing anterior intestinal portal, not more than 1.0-1.5 mm. in width. Of the 24 grafts which were sectioned, 18 were from donors of 35-38 somites and the remaining 6 came from donors of 15, 16, 19, 21, 26, and 29 somite stages. All but 1 of these (21 somites) developed hepatic tissue which was apparently normal, i.e., it corresponded closely to the liver from a normal 11-day old embryo and conformed to the description of the grafted liver tissue given above.

Tissue typically pancreatic in its arrangement has been observed in these grafts, but because the material was fixed in Bouin's solu-

tion, its characteristic components could not be identified by selective staining methods.

In all of these transplants there appear to be a few free granulocytes, especially between the tubules of the liver. They are not localized but are scattered at random, more occurring in some areas than in others.

In addition to the foregoing 24 grafts of liver and gut tissue, 4 grafts of the liver rudiment free from the adjacent gut from donors of 3 days' incubation are included here. In these the hepatic tissue which is formed is identical with that found in grafts of both rudiment and adjoining gut. It is clear that the most normal hepatic tissue occurs in grafts obtained from donors during the third day of development.

4. GRAFTS OF LIVER RUDIMENTS AND TISSUES FROM OLDER EMBRYOS

A total of 41 grafts has been examined histologically and a summary of the results obtained is presented in Table I. Twenty-five of these grafts were obtained from transplants in which hepatic tissue alone was isolated, and in 16 grafts a piece of adjacent gut was included with the liver tissue. The purpose of the two types of isolation was to determine if possible whether the tissue had the same capacity for differentiation in the presence or absence of the gut. In the older embryos, owing to the large size of liver, pieces were transplanted instead of the whole gland. In several grafts of such cases histological examination showed a lack of gall-bladder epithelium.

Twenty-three grafts were examined from donors of 4 days' incubation—13 of them contained hepatic rudiment alone and 10 included liver and gut. The hepatic tissue which differentiates in these grafts is essentially normal. The cells of the hepatic cords, however, are more vacuolated, and mitotic figures are less frequently seen than in the grafts of the younger stages. The sinusoids are apparently more numerous. The fact that mitotic figures do occur, and that the graft is large, shows that growth has not completely ceased. In these transplants there is a slight increase in the number of granulocytes over the number found in grafts of younger stages and in the normal

liver. They occur in masses among the hepatic cords in some areas, others being entirely free of them.

Seven grafts were studied from embryos of 5 days' incubation. Of these, 6 were a combination of liver-and-gut tissue, and 1 was a portion of hepatic tissue alone. All the grafts give evidences of degeneration in the hepatic tissue. The granulocytes, which are now found

TABLE I
SUMMARY OF RESULTS ON LIVER DIFFERENTIATION

DONOR AGE (DAYS)	TOTAL AGE OF GRAFTS (DAYS)	NO. GRAFTS EX- AMINED	HISTOLOGY OF THE GRAFT			
			Liver Tubules	Sinusoids	Infiltration	Necrosis
15-28 somites.	11	19	Normal; mitoses present	Numerous; connected with host blood vessels	Few	None
3	12	4	Normal; mitoses present	Numerous; connected with host blood vessels	Few	None
4	13	23	Some cells vacuolated; few mitoses	Enlarged	Definitely localized	None
5	14	7	Some normal; some abnormal	Enlarged	Throughout grafted tissue	Traces
6	15	11	Nuclei and cell membranes degenerated	Empty	Heavy throughout graft	In extended areas

spread indiscriminately through this tissue, have apparently engulfed the hepatic cells, taking their places in some of the tubules. The result is a transformation of the tubules, ranging from normal ones with clear-cut central lumina and typical cells to abnormal ones with lumina surrounded by cells in all stages of degeneration. The process may even go so far as to leave only a reticulum around the lumen of the hepatic tubule, the cytoplasm and nuclei being entirely gone.

From 6-day donor embryos, 11 grafts of hepatic tissue were examined histologically. The implants are all of considerable size, in-

dicating good growth; but actually the size is apparently due to the presence of large watery vesicles or to gut tissue, the amount of liver tissue being less than the amount originally transplanted. Moreover, the liver tissue is difficult to recognize in most cases, since the hepatic cells are highly degenerate, i.e., the cells, nuclei, and cell membranes have disintegrated, leaving only traces of their outlines in the mesenchyme. The sinusoids have increased in width and contain very few blood corpuscles. The infiltration of granulocytes is very heavy throughout the mass of hepatic cords. On the whole, the picture is one of extensive degeneration. These experiments show that embryonic liver tissue, grafted to the chorio-allantoic membrane, first shows a cessation of growth and differentiation on the eleventh day of its development, and by the fourteenth day its degeneration is complete. From the work of Murphy and others it is known that adult liver will not persist in chorio-allantoic grafts, consequently no further experiments on later stages were attempted.

When gut is included with the liver rudiment or tissue in the graft, the granulocytic invasion is slightly retarded and the vacuoles in the cytoplasm of the hepatic cells are not as numerous as in the cases where gut is absent. Furthermore, it should be noted that in the majority of cases where gut is included in the isolation, gall-bladder epithelium and pancreatic tissue occur and both seem to be in a normal and somewhat active condition.

5. GRAFTS OF PANCREATIC TISSUE FROM DONORS OF 4-19 DAYS' INCUBATION

Since the pancreas is made up of three distinct parts—acini, ducts, and islands—and each part develops at a different time in the embryonic life, it was necessary to use donors which varied in age from 4 to 19 days of development. The pancreas isolated included the ventral primordia up to 7 days of development, at which time the dorsal diverticulum has extended into the same mesenchyme and consequently is also included. A brief summary of the results of these operations will be found in Table II.

The first experiments deal with donors of 4 days' incubation. Before this time, on account of the close connection of the liver, a complete isolation is practically impossible. Almost as common as the

appearance of hepatic tissue in these grafts is the presence of sympathetic ganglia. These are always found in with the tubular tissue of the pancreas and doubtless were included with the original isolation. The earlier attempts to make pure isolations were not always

TABLE II
SUMMARY OF RESULTS ON PANCREAS DIFFERENTIATION

DONOR AGE (DAYS)	TOTAL AGE OF GRAFTS (DAYS)	NO. GRAFTS EX- AMINED	HISTOLOGY OF GRAFT					
			Pancreas			Granu- locytes	Necrosis	Invasion of Connec- tive Tissue
			Islands	Acini	Ducts			
64-90 hours.	11-12	19	More than normal	Normal but no zymo- gen gran- ules	Normal	Few	None	None
4-6....	13-15	13	Still more abundant	Normal but no zymo- gen gran- ules	Normal	Few	None	None
7-8....	16-17	9	Greatest quantity	Normal but no zymo- gen gran- ules	Normal	Few	None	None
8-9½...	17-18½	13	Slight de- crease	Normal but no zymo- gen gran- ules	Normal	Few	None	Notice- able
10-11...	19-20	11	Still greater decrease	Normal but no zymo- gen gran- ules	Surrounding connec- tive tissue increased	Slight in- crease	Trace	More abun- dant
12-13...	21-22	9	Still greater decrease	Normal but no zymo- gen gran- ules	Surrounding connec- tive tissue increased	More abun- dant	Trace	Still more abun- dant
14.....	23	7	Very little	Acini re- duced	Surrounding connec- tive tissue increased	More abun- dant	Trace	Still more abun- dant
15-16...	24-25	10	None	Acini re- duced	Very small	More abun- dant	More ex- tensive	Very ex- tensive
17-19...	26-28	19	None	Very com- pressed	None pres- ent	Maximum number	Maximum amount	Maximum amount

successful since in several grafts gall-bladder epithelium was found and in one case heart tissue was identified.

Comparatively few transplants were studied in which the gut was included with the pancreas, because the presence of the former seemed to make no appreciable difference in the differentiation of the latter. In all grafts the pancreatic tissue is in acinar form, but every section stained with specific stains shows the cells to be devoid of the characteristic zymogen granules, the indicators of secretory

activity. Furthermore, the result is the same regardless of whether a piece (either a half or a third) or the whole of the embryonic pancreas from donors of 10 days or older is grown in a graft. In each case the connective tissue is found invading the pancreatic tissue, necrotic areas are present, and masses of granulocytes are found. On the basis of general experience in chorio-allantoic grafting, however, it seems logical to assume that the smaller piece gives the more typical picture.

In grafts obtained from donors of 4-8 days, the island tissue is so extensive that a section stained by the routine method displays the beta cells with unmistakable clearness, and in the best sections they seem much more abundant than in the normal pancreas. In the majority of these grafts the number of free granulocytes is not appreciably greater than the number in the normal chick pancreas of a corresponding age, where they are more abundant than in at least some of the organs and tissues examined. It is interesting to note that where liver and pancreas occur in the same graft, the granulocytes are more abundant in the former.

Certain changes are seen in the grafts of pancreatic tissue of 8- and 9-day donors. For instance, in several there is a slight but definite decrease in the occurrence of island tissue, and occasionally an increase in the amount of connective tissue between the tubules. This slight increase is apparently significant when considered in the light of observations made on older stages.

In the transplants obtained from donors of 10-14 days, a very noticeable decrease is noted in the amount of island tissue. In grafts of pancreatic tissue from 14-day chicks the islets are entirely lacking in 5 of the 7 cases examined. In the other 2, a few alpha and beta cells in the midst of dense aggregations of granulocytes represent the former endocrine part of the tissue. In some instances the granulocytes are so abundant as to obscure even these few cells. With the decrease of the island tissue there is a simultaneous increase in the number of granulocytes. Also the amount of connective tissue present is much greater in a graft of a 14-day chick than in one from a 10-day donor, showing that this process, too, is a progressive one.

The grafts of pancreatic tissue from donors of 15-19 days are most striking in that no island tissue is to be found in any of them.

The tissue is lobulated, but only because large amounts of connective tissue have developed between the tubules, so that they are greatly compressed and reduced to a size much smaller than normal. The granulocytes are present in great masses, typically occurring around and between the compressed acinar-like tissue.

GENERAL DISCUSSION

These experiments show that the hepatic rudiment of the chick embryo has the capacity for independent differentiation into specific hepatic tissue. Hepatic rudiments from donors which have been incubated up to 3 days differentiate normally into cells of a characteristic nature, these cells forming tubules that are separated by many typical vascular sinusoids. The outstanding difference between the hepatic tissue of the graft, and that of a control embryo of a corresponding age, is its arrangement. In the graft it is always in an irregular mass with no organization into lobes. The form and structure of the hepatic tissue in these early grafts is the same as that described in the paper of Willier and Rawles (1931a, p. 90) on the developmental relations of heart and liver. In all grafts in which liver tissue attains less than a total age of 12 days, it is histologically normal. Beginning at about this time, a change is indicated by an increase in the size and number of vacuoles occurring in the hepatic cells. This process is slightly retarded if the piece of adjacent gut is included with the liver rudiment in the isolation. Coincident with this change in the hepatic cells is a noticeable increase in the numbers of free granulocytes appearing in occasional areas of the liver tissue. The grafts from older donors show these changes even more definitely, and, finally, when they have reached a total age of 14 days, the hepatic cells have undergone such an extensive degenerative process, and the granulocytes occur in such great numbers throughout the graft, that it is no longer possible to distinguish the tubules.

The reason for the onset of the degenerative process is not known, but the fact that it occurs regularly at a certain time in the differentiation of the gland in the graft suggests that a specific change of some sort acts at this time, which renders it incapable of further development outside of its natural and original environment. Other

workers in this field have noticed a similar behavior of the liver tissue. In a personal communication Willier states that in chorio-allantoic grafts of liver tissue from hatched chick donors, only the bile ducts remain in the midst of an intense infiltration of leucocytes, the liver cells apparently having undergone disintegration. Also the descriptions by Murphy (1916, p. 2) and Willier (1924, p. 90) of this reaction in the grafted liver tissue indicate that the result is a constant one.

This peculiar degeneration of liver tissue in the graft may be correlated with the biochemistry of the organ. From the work of Hanes (1912), Heaton (1926), Needham (1927), and others, it has been shown rather conclusively that on the eleventh day, and shortly thereafter, there is a physiological change of special significance taking place in the developing chick. In tissue-culture studies of chick liver, Heaton (1926) noticed that the outgrowth of embryonic liver of 11 days' development changed morphologically and physiologically from epithelial cells to cells with reactions like those of fibroblasts. This is natural since up to the present time it has been reported that all cells kept in culture for a time tend to look like fibroblasts, but the significant and unexplained feature is that this change occurs on the eleventh day and not before. Furthermore, Needham (1927) has shown by means of graphs and descriptions based on chemical analyses of whole embryos that there is a distinct change in the energy source from protein to fat, and a marked storage of glycogen commencing on the eleventh day of incubation. Also Hanes (1912) has noted a substitution of the phosphorized fats by cholesterol esters in the liver, on the fourteenth day of development, which is shortly after the physiological change noted by Needham. The findings of all these workers seem to suggest a significant correlation between the degeneration in grafted tissue and certain physiological changes occurring in normal hepatic tissue at approximately the same time.

The loss of capacity to maintain a high state of differentiation on the part of the tissue in the graft has been noted by others. Hiraiwa (1927), working with rat embryos, found that very early in development the tissue from the central nervous system ceased to develop in a chorio-allantoic graft. No mention was made of a species speci-

ficity, but it might possibly have been an explanation in this case. Sandstrom (1930), working with duck-kidney tissue grafted to the chorio-allantoic membrane of a chick, obtained a similar result and attributed it to an effect of species specificity. His later observations, however (1932), in which he grafted duck tissue to duck host, seem to point to a lack of function of the tissue in the graft. E. A. Hunt (1931) has found the same thing true of grafted skeletal muscle. In her case there could be no question of a species specificity because the donors and hosts were both chick material. Likewise in the present work, the degeneration of the grafted liver tissue may be due to a lack of function in the graft. The recent work of Doljanski (1931) on chick-liver tissue cultured in a specially treated artificial medium may in a way lend support to this interpretation of a lack of function. He cultured the liver tissue in a medium which had been so treated that the fibroblasts could not live. In this medium the liver cells grew for months as so-called pure cultures of specific hepatic cells, and Doljanski attributes this to the fact that the liver cells, being functioning entities in themselves, could manufacture that which was lacking in the medium and so continued to exist and grow. Possibly the ability to function was what kept the cells in the culture alive, and conversely in the presence of a host which is functioning there is no stimulation of the grafted tissue which as a result degenerates.

The problem of differentiation of the pancreatic tissue in grafts is a more complicated one since both the exocrine and endocrine components are represented. In grafts taken of the rudiment stages of the gland up to those obtained from donors of 8 days, the tissue appears to differentiate normally. The cells of the tubules are arranged in groups as they are in the secreting acini found in the normal pancreas. However, with the aid of specific stains it is found that the apparently normal groups of acinar-like cells, even from the older donors, completely lack the zymogen granules. A comparison of these results with the normal condition in which zymogen granules are first noted on the tenth day indicates a suppression of the secretory function.

That this apparent suppression of zymogen production is due to the isolation of the gland from an active gut is suggested by the

work of Grauer (1926). He ligated the pancreas of the rabbit and found as a result a period of retrogression at first. After this period secretory activity is apparently renewed, but since no outlet is afforded, absorption of the acini begins and by the end of 19 days they are completely replaced by trabeculae of connective tissue. In chorio-allantoic grafts of pancreas, an effect similar to ligation is obtained, for the gland is growing independently of a functioning gut system and this may serve to suppress the secretory function of the acini. A piece of the gut grafted with the pancreas does not change the reactions of that gland in the least, and this is perhaps to be expected since only a piece of the gut is present and so cannot be functioning as in the normal.

In grafts of the pancreatic rudiment from embryos of 3-9 days' incubation the island tissue is far more abundant than in the normal pancreas of embryos of corresponding ages. For instance, the pancreas rudiment after it has attained a total age of 12 days in the graft is full of island tissue, whereas in the normal embryo of this age the alpha and beta cells are just appearing. In grafts of pancreatic tissue from older stages, however, a gradual but definite decrease in the number of islands present is seen. At first, in grafts obtained from donors of 10 and 11 days, this decrease is slight, but in 5 out of the 7 cases examined from 14-day donor embryos, there is a total lack of alpha and beta cells and none of the grafts from donors of 15-19 days' incubation shows any islet cells whatever.

The acceleration of island production in the graft is similar to an increase of island tissue noted by Grauer (1926) when the ducts of the pancreas of the rabbit were ligated. However, the decrease and disappearance of these cells in the later stages is in marked contrast to his statement that the island tissue remains growing for months after ligation (p. 251). In the rabbit the islands persist apparently for the reasons that (*a*) their secretion is poured into the bloodstream through normal channels and (*b*) they are the only source of secretion in the animal. In the grafts, although the blood supply is intact, the island tissue gradually decreases as the age of the donor increases, so the phenomenon must be traced to some other cause.

The unusual condition, whereby the amount of island material of grafted embryonic pancreatic tissue undergoes an initial increase

followed by a progressive decrease, is as yet unexplained. The acceleration of production which occurs when younger tissue is transplanted may be interpreted as a response to an approaching functional need in the host during the first few days after the graft was made, since in the normal embryo the islets do not begin to appear before the twelfth day and are not well formed until about the fifteenth day. For example, a rudiment of a 4-day donor embryo develops in the graft for a minimum of 3 days before the host island tissue begins to secrete, as is indicated by the appearance of secretion granules in the alpha and beta cells. The gradual decrease and final disappearance of the island tissue in the grafts, noted as the age of the donor increases, is perhaps accounted for by the onset of island activity in the host. This situation should be investigated further by grafting pancreatic rudiments from early donors to hosts in which the endocrine function has already appeared. If the active islands of the host are the influencing factors, then no islands would be expected to appear in the graft. Similarly, further data may be obtained from a study of the pancreatic tissue of older donors, in grafts removed just before the endocrine function of the host begins. The island tissue of such grafts on the basis of this interpretation would be expected to show continued growth and differentiation and probably an increase, as is seen in the grafts from younger donors.

On the other hand, an important factor influencing the observed histological changes may be the age of the pancreatic tissue of the donor at the time of its implantation. Since all of the grafts are on the host for the same length of time and so are equally subjected to its endocrine activity (from the twelfth to the eighteenth day of incubation), it is possible that the age of the grafted tissue rather than the hormone of the host is responsible for the results obtained in the grafts. Further evidence for this interpretation may be obtained from the experiments stated in the preceding paragraph in which the functionally differentiated island tissue is removed from the chorio-allantoic membrane before the host islands begin to secrete. If degeneration rather than hypertrophy of the island tissue is found in the grafts, the importance of the age factor would be indicated. This interpretation is in line with the observations made previously, that liver tissue differentiates normally until it reaches a stage in

which it is functionally differentiated, at which time it degenerates — a situation which has also been described for the metanephros (Sandstrom, 1932).

In the present grafting experiments both the liver and the pancreas rudiments are incapable of forming organs of normal shape. In each case the grafted tissue occurs in irregular masses, no lobes or lobules being formed, and often the tissue occurs in a number of groups. This happens whether the rudiment alone or in conjunction with a piece of gut tissue is transplanted, and also whether the whole gland or only a fragment of it is grafted. The failure of the normal form of the organs to appear in the graft introduces an interesting question. Pancreatic grafts obtained from older donors often present a distinct appearance of lobulation, noticeable macroscopically as well as histologically. However, this seems to be due rather to the thickened trabeculae of connective tissue in and around the glandular masses than to a normal differentiation.

Willier (1931a, p. 298), in a discussion of this problem with respect to the liver, suggests that "the absence of a septum transversum and of the coelom (the liver in the graft is surrounded by mesenchyme except when and where attached to the heart) are possible factors necessary for the determination of form in the liver." Murray and Huxley (1925), in studying grafts of chick tissue, state that self-differentiation is essentially a chemical process, which leads to the production of specific tissues in definite amounts; but the assumption of particular morphological form must be largely determined by physical (i.e., mechanical) influences. Accordingly, it may be presumed that such influences are lacking in these grafts.

Beginning on the eleventh day of hepatic and about the twentieth day of pancreatic development, there is an infiltration of granulocytes in these tissues, reaching the greatest concentration on the fifteenth day in the former and the twenty-eighth day in the pancreas. This progressive increase in granulocytes parallels the degeneration of the hepatic tissue, or, in the case of the pancreas, the disappearance of island tissue. A similar infiltration of leucocytes has been noted by Murphy (1916, p. 3) and Willier (1924) in grafts of liver tissue, and by Danchakoff (1918) in grafts of the spleen. This process may go so far as to extend to certain organs of the host em-

bryo, e.g., spleen and liver, as described by Willier. On the other hand, Willier (1927 and in a personal communication) reports that commonly such infiltration and degeneration does not occur in grafts of the Wolffian body and other embryonic tissues. Neither Hoadley (1925) nor Hunt (1931) mentions it in any of their experiments on grafting of various embryonic areas. It is thus evident that a definite correlation exists between the invasion of the granulocytes and the process of degeneration and necrosis—that is, whenever degeneration sets in, granulocytes appear.

SUMMARY

1. This problem is an attempt to analyze the growth and differentiation of liver and pancreatic primordia and tissues of chick embryos as tested in chorio-allantoic grafts. The host embryos were usually 9 days old at the time of implanting; the grafts were permitted to grow for either 8 or 9 days.

2. In the earliest grafts the isolations were of three sorts: (*a*) a piece of the floor of the gut large enough to include the hepatic diverticula, (*b*) a piece of the roof of the gut including the dorsal pancreatic rudiment, or (*c*) a ring of gut tissue including the rudiments of both glands. From donors of 3 days or more, the gland was either taken alone or included with a small portion of the adjoining gut tissue.

3. The isolated hepatic rudiment of embryos is at first capable of independent differentiation. In all such grafts the liver appears as an irregularly shaped mass of tubules and sinusoids, histologically normal, but lacking any organization into lobes. An epithelial vesicle identified as the gall bladder occurs in many of the grafts and appears to be normal. Beginning on the twelfth day of development, a degeneration of the hepatic cells of the graft sets in, which by 15 days has become so extensive that many of the hepatic cells are completely disintegrated and there is an infiltration of granulocytes throughout the tissue of the graft.

4. Pancreatic rudiments from donors of approximately 3 days and older have the capacity to undergo self-differentiation within certain limits. (*a*) The Islands of Langerhans, identified by their characteristic alpha and beta cells, appear in abundance throughout the grafts

from donors up to 9 days' incubation. Grafts made from older donors show progressively fewer numbers of islands until in those obtained from 15-day donors there is none. (b) The acinar-like tissue appears normal, but when stained selectively for zymogen granules, it always gives negative results, indicating that there is probably no exocrine secretion in the grafted tissue.

5. Paralleling the degenerative processes noted above is an increase of granulocytes, the significance of which is not known.

6. The cessation of growth and subsequent degeneration of liver and pancreatic tissues in grafts to the chorio-allantoic membranes of host embryos apparently coincides with the beginning of functional activity. Their inability to function in the graft leads to their degeneration.

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